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The extent of chromosomal aberrations induced by chemotherapy in non-human primates depends on the schedule of administration

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Abstract

We utilized a non-human primate model, the rhesus monkey (*Macaca mulatta*), to quantitate the extent of chromosomal damage in bone marrow cells following chemotherapy. Thiotepa, etoposide, and paclitaxel were chosen as the chemotherapy agents due to their distinct mechanisms of action. Chromosomal aberrations were quantitated using traditional Giemsa stain. We sought to evaluate the extent to which genotoxicity was dependent on the schedule of administration by giving chemotherapy as either a bolus or a 96 h continuous infusion. Neutropenia and areas under the concentration curve (AUCs) were monitored to ensure comparable cytotoxicity and dose administered. At least 100 metaphases were scored in each marrow sample by an investigator unaware of the treatment history of the animals. All three drugs produced a statistically significant higher percentage of abnormal metaphases following bolus chemotherapy ($p < 0.0001$, $p = 0.0015$ and $p < 0.0001$ for thiotepa, etoposide and paclitaxel, respectively). We conclude that infusional administration of thiotepa, etoposide and paclitaxel is less genotoxic to normal bone marrow cells than is bolus administration. These results suggest infusional regimens may be considered where there are concerns about long-term genotoxic sequelae, including secondary cancer, teratogenicity, or possibly the development of drug resistance. We believe this approach provides a reproducible model in which drugs and eventually, regimens can be compared.

Keywords: Drug resistance; Chromosomal damage; Genotoxicity; Secondary malignancies; CIV chemotherapy

Abbreviations: AUCs, areas under the concentration versus time curve; CIV, Continuous intravenous infusion

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1. Introduction

As advances continue to be made in the successful treatment of cancer, attention is being directed at the complications of chemotherapy. The occurrence of secondary leukemias and the teratogenic potential of chemotherapy have long been recognized, but not systematically addressed [1–3]. Extensive data exists documenting the ability of chemotherapeutic agents to cause chromosomal damage [4–16]. Such damage is likely responsible for secondary leukemias and teratogenicity. However, prospective studies have not been performed to evaluate the frequency and severity of chromosomal damage following treatment with chemotherapy, nor have strategies been investigated which might reduce its occurrence. Studies performed three decades ago showed frequent gross chromosomal aberrations in normal bone marrow cells and circulating peripheral blood lymphocytes following exposure to chemotherapeutic agents [1]. Karyotypic changes were observed both when normal bone marrow cells were cultured ex vivo in the presence of therapeutic concentrations of anti-neoplastic drugs as well as in the bone marrow and peripheral blood cells harvested from cancer patients 4–6 days after commencing a cycle of chemotherapy [2]. Given these findings, it is not surprising that subsequent studies have reported the occurrence of secondary malignancies in patients treated with chemotherapy as well as following autologous and allogeneic bone marrow transplantation [3–11].

In addition to myelotoxicity, germ cell damage and teratogenic effects have also been observed following chemotherapy [12]. However, systematic studies have not been performed and the existing data can best be characterized as incomplete. A 1978 study showed that chromosomal aberrations affecting embryonal tissue occurred most frequently following VP-16 or VM-26 [12]. In addition, congenital malformations have been linked to anti-metabolites, including methotrexate and alkylating agents. Treatment with alkylating agents during the first trimester can result in genetic damage, with four cases of fetal malformations reported in 53 patients receiving cytostatic drugs during the first trimester [13]. The mutagenicity of alkylating agents is related to their ability to form cross-links and/or transfer an alkyl group to form monoadducts in DNA [14,15] and their clastogenic effects include aneuploidy of autosomes and sex chromosomes.

Similarly, the frustrating problem of acquired resistance to chemotherapy, although frequently encountered, has not been addressed from the viewpoint of preventing its emergence. For the multidrug resistance gene, *MDR*-1, there is enough evidence to indicate how acquired resistance to chemotherapy can occur as a result of chromosomal rearrangements that result in hybrid messages wherein, the expression of *MDR*-1 is under the control of a second gene that is expressed endogenously at much higher levels [17–20].

Thus chromosomal rearrangements can result in secondary malignancies, teratogenic effects, or acquired drug resistance. However, the role of conventional chemotherapy in this has not been prospectively evaluated. The evidence indicates that many chemotherapy drugs cause acute, gross chromosomal damage as well as subtler heritable changes [21–23]. We utilized a non-human primate model to determine whether the extent of chromosomal damage following the administration of chemotherapy could be modulated. Because of their close phylogenetic proximity to humans in the evolutionary hierarchy, non-human primate models have traditionally been recommended for genotoxicity assays, pre-clinical evaluation of chemotherapy agents and as models for assessing the efficacy of human gene therapy protocols [24–29] though rodent models are utilized as a screen prior to primate studies. We compared the genotoxic effect of two different schedules of administration, bolus versus 96 h infusion, on the metaphase chromosome spreads of normal non-human primate bone marrow. For three drugs representing three different classes, we observed less chromosomal damage following infusional drug administration despite comparable pharmacokinetics and neutropenia. We conclude that infusional regimens may be less genotoxic.

2. Methods

2.1. Non-human primate model

The National Cancer Institute Animal Care and Use Committee (ACUC) approved the protocol used in the conduct of this study. The animal procedures were conducted in accordance with the United States Government "*Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and*

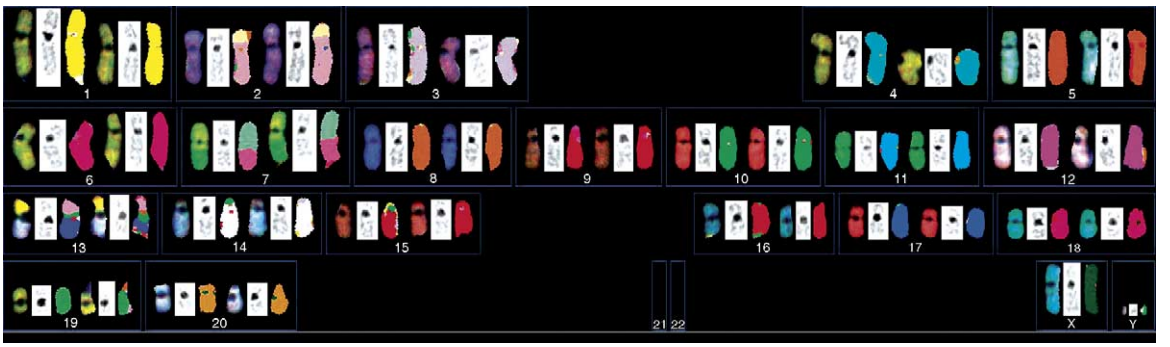


Fig. 1. Spectral karyotyping (SKY) using human paints in rhesus monkey metaphases (42,XY). Apparent translocations at monkey chromosomes 2, 7 and 13 are due to repackaging of chromosomal contents from counterparts in human chromosomes 2, 14, 15, 21 and 22. Human chromosome 2 exists as two chromosomes (i.e. Chromosomes 9 and 15) in a rhesus monkey metaphase.

Training" and the "*Guide for the Care and Use of Laboratory Animals*". We used the Rhesus monkey *Macaca mulatta* species. Their karyotype is well characterized by conventional banding [28,29] and their close proximity to humans has been shown using human probes in spectral karyotyping (Fig. 1). The animals were procured from the NIH non-human primate recycle pool. Young males, weighing 8–10 kg, were selected. Prior participation in other experimental studies was allowed with the exception of those involving chronic drug administration, radiation or chemotherapy. The animals were housed in individual cages, in a social housing environment. We planned to sedate the animals for all procedures, and recognized that this can be accompanied by a period of decreased oral intake. Therefore, care was taken in scheduling to minimize the number of procedures conducted and to avoid multiple procedures on successive days. Study design is outlined in Fig. 2. Chairs and collar restraints were used after training the animals in order to accomplish the daily reloading of the infusion pump, and blood sampling.

2.2. Intravenous access

All animals had two single lumen subcutaneous access ports placed under general anesthesia at least 2

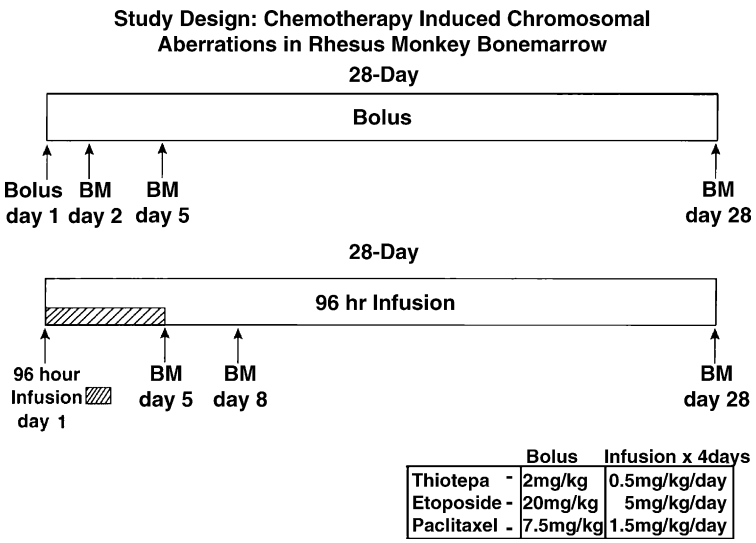


Fig. 2. Study design.

weeks prior to the start of the study (Instech; http://www/instechlabs.com/ports.html). Subcutaneous tunneling was done for placement of both ports at sites over the dorsal regions of the animals. One catheter was placed in the jugular vein by tunneling subcutaneously from the scapula. This catheter was used for administration of chemotherapy. A second catheter was placed in the femoral vein by tunneling subcutaneously from the area above the posterior iliac crest. This catheter was used for blood sampling and the administration of drugs other than chemotherapy. The anesthesia used consisted of induction with Telazol (2–6 mg/kg IM), followed by intubation and maintenance of anesthesia with inhalation of 0.5%–1.5% Isoflurane with a mixture of nitrous oxide and oxygen. Post-operatively, the animals received Cefobid 20 mg/kg IM twice daily for 3 days. They also received either Ketoprofen 0.5–1.0 mg/kg or Buprenorphine 0.03 mg/kg IM twice daily as needed for pain.

2.3. Drug doses, schedules and mode of administration

Dose calculations were performed using a nomogram (Fig. 3) to convert the dose from man to rhesus monkey. For example, when calculating the doses for a 10 kg animal, the drug dose administered to humans as mg/m^2 body surface area (BSA) needs to be divided by a factor of 20 to determine the dose/kg for the animal. Thiotepa, etoposide phosphate and paclitaxel were chosen as the chemotherapy agents due to their distinct mechanisms of action, i.e. thiotepa is an alkylating agent, etoposide is a topoisomerase II inhibitor and paclitaxel is a tubulin polymerizing agent. All drugs were administered either as a bolus injection over a period of five to ten minutes in case of thiotepa and etoposide phosphate and sixty minutes for Paclitaxel; or as a continuous infusion over 96 h for all three drugs. Drug doses were as follows: (1) Thiotepa, 2 mg/kg as bolus and 0.5 mg/kg/day × 4 day CIV (continuous intravenous infusion); (2) etoposide phosphate, 20 mg/kg as bolus and 5 mg/kg/day × 4 day CIV; and (3) paclitaxel, 7.5 mg/kg as bolus and 1.5 mg/kg/day × 4 day CIV. The total paclitaxel dose for the 96 h infusion of 6 mg/kg was chosen based on experience with humans demonstrating greater myelosuppression with 96 h schedules. A Pegasus Pump, Model LAS-10150 from Instech Solomon Scientific, Plymouth Meeting, PA (info@solsci.com) was used to administer the infusions. Bags containing drug were changed every 24 h for the 96 h infusion. The pumps were placed on the back of the animals, and were held in place by custom-

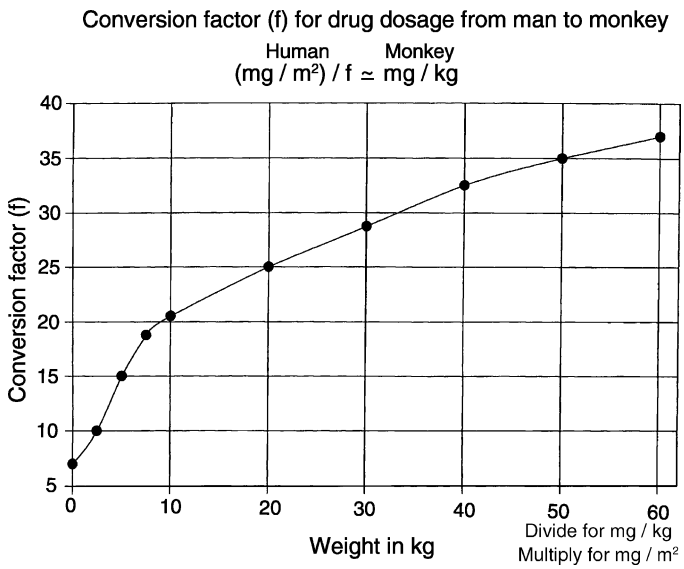


Fig. 3. Nomogram used for calculation of dose of chemotherapy agents in non-human primate models.

made jackets with a pouch that covered the pump so as to provide an additional barrier without impairing animal movement.

2.4. Monitoring before, during and after chemotherapy infusions

Pre-therapy evaluation included a bone marrow aspirate and blood sampling for complete blood count with WBC differential, renal and hepatic profiles. Animals were closely monitored for any signs of distress, pain, discomfort or infection following surgery and prior to starting the chemotherapy infusion. Animals were also monitored for signs of distress during chemotherapy administration and, when needed, hydration and 0.1 mg/kg of the anti-emetic prochlorperazine were administered. Following the administration of chemotherapy, animals were monitored for signs and symptoms of chemotherapy-related toxicity including allergic reactions and hypotension, and had serial blood counts and chemistries performed.

2.5. Study groups and schedule of blood sampling

Based on preliminary data it was decided that a minimum of six animals was needed to achieve adequate statistical power. Each animal first received the study drug by either a bolus or a 96 h infusion. After a recovery period of at least 6 weeks, they were crossed over to the alternate regimen: bolus to 96 h infusion and 96 h infusion to bolus. In this way each animal served as its own control, and which schedule was administered first varied from animal to animal. In ten cases bolus administration was done first, and in eight cases infusion administration was done first. After a recovery period of at least 6 weeks, but often longer, a different drug was administered to most of the animals. One animal received all three drugs, while seven animals received two drugs, and one animal was treated with only one drug. The animals were monitored for acute drug toxicity by close clinical observation and laboratory profiles including complete blood counts and biochemistry studies to assess renal and hepatic function. Blood samples were collected on days 1, 5, 8 and 15 of each cycle (day 1 = start of chemotherapy). In addition all animals had blood sampled for pharmacokinetic studies on the following schedule: bolus cycles at 0 min, 60 min, 120 min, 240 min and at 24 h; infusion cycles at 24 h, 48 h, 72 h, and 96 h during bag changes.

2.6. Bone marrow sampling and processing

Bone marrow aspirations were performed prior to the administration of chemotherapy as well as on days 2, 5, and 28 in the bolus cycle and days 5, 8, and 28 in the 96 h infusion cycle. These time points were chosen to ensure sampling of bone marrows only after complete exposure to a cycle of chemotherapy, and to obtain a baseline bone marrow aspirate prior to the administration of any chemotherapeutic agent (Fig. 2). Bone marrow samples were obtained following proper sedation using either 2–6 mg/kg zolazepam–tiletamine or 0.03 mg/kg buprenorphine IM. Two to four milliliters of bone marrow was aspirated using a pediatric bone marrow needle (11 gauge) from alternate iliac crests. Each bone marrow sample was processed in two aliquots. Direct harvesting of metaphases on the same day was done by immediately collecting part of the bone marrow aspirate into RPMI 1640 culture medium containing 0.05 μg/ml colcemid. Another aliquot was harvested after culturing the cells for 96 h in RPMI 1640 medium with 20% fetal calf serum and 1 μg/ml Staphylococcal enterotoxin A (Sigma–Aldrich Chemical Co., St Louis, MO) as a mitogen [30]. Metaphase spreads were obtained by adding 0.05 ml colcemid (10 μg/ml stock) to a final concentration of 0.05 μg/ml for 2 h followed by hypotonic shock and fixation in glacial acetic acid and methanol (1:3) as previously described [31]. The chromosome spreads of all samples were evaluated using conventional Giemsa staining. A minimum of 100 metaphases per sample was scored by light microscope examination. All sample slides were coded following harvest by one investigator while a second investigator unaware of the code scored them all, blind to identification of the animal, the schedule of administration or the time of harvest. Both structural and numeric aberrations were scored for breaks, fragments, rings, gaps and complex aberrations.

2.7. Statistical methods

The fractions of abnormal metaphases identified in both the direct and mitogen-stimulated sam-

ples, as well as overall, were compared between the bolus and infusion schedules of drug administration for each drug, using an exact stratified Cochran-Armitage test as implemented by StatXact 4 [57]. Comparison between the AUC of bolus and infusion schedules was done using a paired t-test after confirming normality of the paired differences. The differences in the ANC values between pre-bolus and bolus nadirs, and pre-infusion and infusion nadirs, were found to be at least somewhat dependent on the pre-treatment level. To properly address this, the relative differences ((bolus-pre)/pre) or (infusion-pre)/pre) were calculated, and the resulting values were evaluated for being significantly different from zero by the one-sample *t*-test, after confirming reasonable consistency with normality. All *p*-values are two-tailed and have not been corrected for multiple comparisons.

2.8. Pharmacokinetics

Plasma samples were frozen at −70 °C until drug analysis. Thiotepa (1), etoposide (2) and paclitaxel (3) were measured using previously described assays [32–34]. Briefly, thiotepa was extracted from plasma into ethyl acetate containing 1 μg/ml diphenylamine (Sigma–Aldrich Chemical Co.) as an internal standard. Drug was separated and quantitated using a Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen-phosphorous detector (Agilent Technologies, Palo Alto, CA) and a SPB-5, 30 m, 0.22 mm fused silica capillary column (Supelco, Bellfonte, PA) with helium as the carrier gas. Thiotepa concentrations were calculated from the ratio of the peak area of thiotepa to internal standard using a Hewlett Packard 3393A integrator. Etoposide concentrations were determined by reverse phase HPLC. Plasma samples for determination of total drug were extracted with chloroform. The organic phase was collected after centrifugation and evaporated to dryness under nitrogen. The analyte was separated using a Waters 2690 HPLC system (Waters Corp., Milford, MA), a Zorbax 5 μm, 4.6 mm × 25 cm phenyl analytical column (Agilent Technologies) and a mobile phase consisting of water:methanol:acetonitrile:glacial acetic acid (45.5:25:25:0.5, v/v/v/v) with 1.81 g/l tetramethylammonium hydroxide pumped at 1.0 ml/min. Quantitation was performed by an ESA Coulochem II electrochemical detector equipped with a model 5011 analytic cell (ESA, Chelmsford MA) at 550 mV. Paclitaxel was assayed using an isocratic reverse phase HPLC method. Plasma samples were prepared by cyano solid-phase extraction and separation achieved by injection onto a C84 μm, 8 mm × 10 cm NovaPak column (Waters Associates, Milford, Mass.) using a mobile phase of acetonitrile:methanol:10% phosphoric acid:water (10:60:1:29, v/v/v/v) at 1.0 ml/min. Detection was by UV absorbance at 227 nm. Paclitaxel concentrations were calculated from the ratio of the peak height of paclitaxel to internal standard (docetaxel). For bolus administration of drug, areas under the concentration × time curves were calculated by the linear-trapezoidal method [32] and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. For continuous infusion drug administration, areas under the concentration × time curves were calculated by averaging the sample concentrations measured for the time points drawn near 24, 48, 72 and 96 h and multiplying by the length of infusion.

3. Results

A total of 36 cycles of chemotherapy were administered to 9 animals: 18 bolus cycles and eighteen 96 h infusions. Six animals were treated in each drug arm. The data from 32 cycles of chemotherapy are included in the analysis. Data from one animal receiving thiotepa is not included in the analysis because the infusion dose was not completely administered. In addition, data from the first animal receiving etoposide phosphate is not included because this animal received a dose twice that of all other animals. This dose resulted in prolonged pancytopenia and delayed recovery of bone marrow function, and was reduced by 1/2 for all subsequent animals. No animal received chemotherapy with an interval of less than 6 weeks between doses. One animal received all three drugs, while seven animals received two drugs, and one animal was treated with only one drug. For each drug cohort, all animals received drug by both bolus and infusion and thus served as their own controls. In 10 cases bolus administration was done first, and in 8 cases infusion administration was done first. One animal that had previously received bolus and infusional thiotepa and etoposide died following the paclitaxel infusion, during the period of neutropenia. Au-

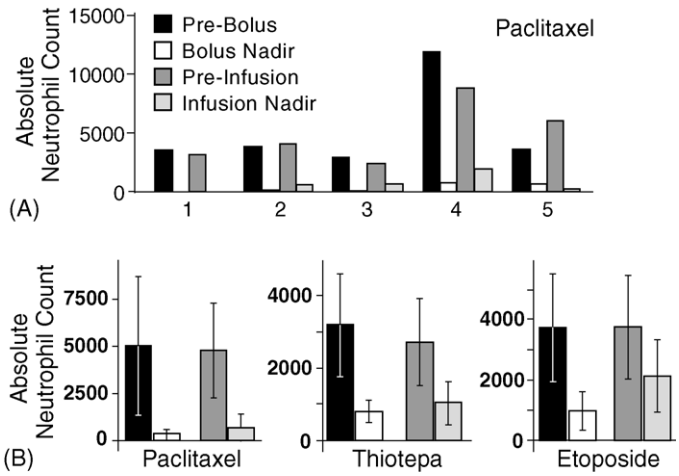


Fig. 4. Neutrophil counts obtained for the animals on study. (A) Neutrophil counts in the five animals receiving paclitaxel. Pre-treatment values obtained prior to the bolus administration (pre-bolus) or prior to the start of the infusion (pre-infusion) are compared with the lowest count recorded after administration of chemotherapy for that cycle (Bolus Nadir and Infusion Nadir). Nadir values bolus were 120, 107, 68, 685, and 645. Nadir values for infusion were 24, 549, 668, 1880, and 151. (B) Neutrophil counts for the group of animals receiving each drug.

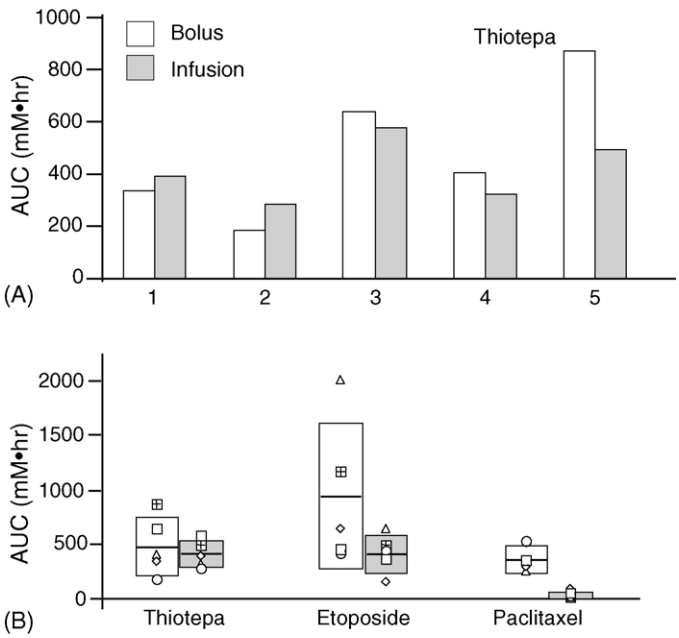


Fig. 5. Area under the concentration curve (AUC) obtained for the animals on study. (A) AUCs in the five animals receiving thiotepa. Values calculated after bolus drug administration are compared with those calculated following infusional administration. (B) AUCs for the group of animals receiving each drug. The vertical line represents the mean value, while the box delineates the standard error. Each animal is identified by a different symbol.

topsy revealed evidence of liver necrosis, the etiology of which was uncertain. Screening liver function tests in this animal had been normal. No other animal experienced a fatal complication. In one animal a catheter infection required parenteral antibiotics and catheter revision. No other serious infections were encountered.

To ensure that equitoxic doses of chemotherapy were administered, absolute neutrophil counts were monitored before the start of chemotherapy and on days 5, 8, and 15 of each cycle, with day 1 being the day of bolus administration or start of infusion. Fig. 4 summarizes the neutrophil counts obtained for the animals on study. Data are presented in panel A for five individual animals receiving paclitaxel and in panel B for the group of animals receiving each drug. The pretreatment values obtained prior to the bolus administration (pre-bolus) or prior to the start of the infusion (pre-infusion) are compared with the lowest count

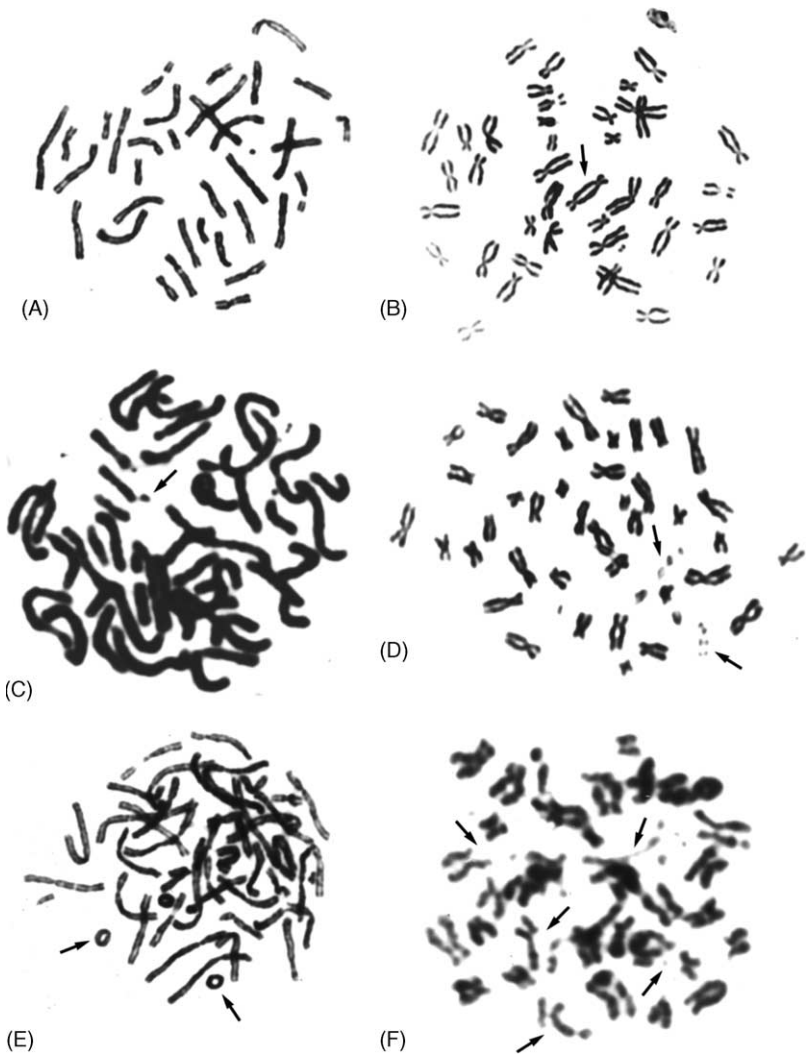


Fig. 6. Examples of chromosomal damage scored in metaphase spreads following Giemsa staining. Arrows in the individual panels identify the different cytogenetic abnormalities. Panel A (normal); panel B (dicentric); panel C (chromatic fragment); panel D (complex aberration showing breaks and fragments); panel E (rings); panel F (multiple breakages and fragments). Representative examples of breaks, fragments, rings, gaps and complex aberrations are shown in the figure.

recorded after administration of chemotherapy for that cycle (Bolus Nadir and Infusion Nadir). In no case did serial values suggest there was a period of prolonged neutropenia in any animal. Within the constraints of the sampling schedule, comparable neutropenia was observed with both bolus and infusion for all three drugs, suggesting that the doses chosen for the bolus and infusion regimens were comparably myelotoxic. The statistical significance of relative differences in ANC between pre- and nadir- evaluations were as follows: thiotepa: $p = 0.0004$ for bolus, $p = 0.0011$ for infusion; paclitaxel: $p < 0.0001$ for bolus, $p = 0.0004$ for infusion; etoposide: $p < 0.0001$ for bolus; $p = 0.0088$ for infusion. Thus, in each case, the nadirs were significantly lower than the pre-administration values. In addition, the area under the concentration versus time curve (AUC) was determined to assess actual drug exposure. Comparable AUCs were anticipated for thiotepa ($p = 0.43$) and etoposide ($p = 0.099$), but not for paclitaxel—with limited data for paired comparison, given the known saturable kinetics previously demonstrated in humans receiving paclitaxel. Fig. 5 summarizes the AUCs obtained for the animals on study. Panel A presents the data in individual animals receiving thiotepa, while panel B summarizes the data for the group of animals receiving each drug.

For these regimens that were comparably cytotoxic, we sought to determine the extent of chromosomal damage as measured by scoring metaphase spreads following Giemsa staining. To do this, metaphases were harvested immediately after bone marrow aspiration (direct) or after culturing the bone marrow cells with mitogen (mitogen-stimulated). This approach was chosen so as to examine all cells immediately, and also to examine replication competent cells after 96 h of culture. Examples of breaks, fragments, rings, gaps and complex aberrations scored in some representative metaphases are shown in Fig. 6. All Rhesus monkeys used in the study have a normal 42,XY karyotype determined by a pre-chemotherapy marrow as all animals used in the study were male. Many morphologically "difficult" metaphases were included in the scoring and labeled complex karyotypes or sticky metaphases as were observed earlier in work cited [1]. In fact metaphases included in Fig. 6 were similar to the metaphases reported in humans following chemotherapy exposure. Chromosome numbers in a metaphase were successfully counted in almost 75% of metaphases and aneuploidies were noted. The percentage of abnormal metaphases scored following bolus and infusion chemotherapy are summarized in Table 1 and depicted graphically in Figs. 7 and 8. Fig. 7 shows

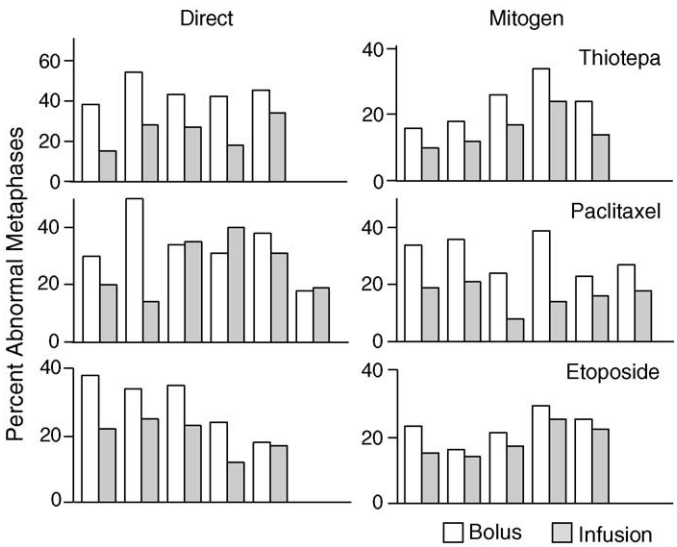
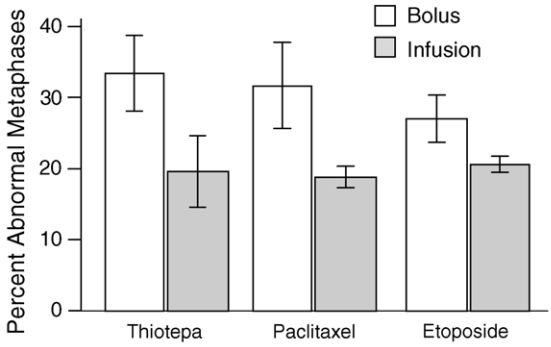


Fig. 7. Tabulation of percent abnormal metaphases found in the bone marrow sample from each individual animal. For each drug, the panel on the left demonstrates the results obtained when metaphases were scored in samples harvested directly after bone marrow aspiration. The panel on the right summarizes the data examining metaphases harvested from samples cultured in staphylococcal mitogen for 96 h.

Table 1
Abnormal metaphases following chemotherapy exposure

	Animal	1	2	3	4	5	6
Thiotepa bolus							
Direct	# Abnormal	43	58	63	20	26	–
	Total scored	114	107	146	47	58	–
	% Abnormal	38	54	43	42	45	–
Mitogen	# Abnormal	24	17	34	26	24	–
	Total scored	148	92	100	100	100	–
	% Abnormal	16	18	34	26	24	–
Thiotepa infusion							
Direct	# Abnormal	8	31	29	9	17	–
	Total scored	53	111	105	48	50	–
	% Abnormal	15	28	27	18	34	–
Mitogen	# Abnormal	16	15	30	17	14	–
	Total scored	154	125	124	100	100	–
	% Abnormal	10	12	24	17	14	–
Etoposide bolus							
Direct	# Abnormal	30	26	45	10	12	–
	Total scored	78	76	128	41	65	–
	% Abnormal	38	34	35	24	18	–
Mitogen	# Abnormal	23	14	21	29	25	–
	Total scored	100	87	100	100	100	–
	% Abnormal	23	16	21	29	25	–
Etoposide infusion							
Direct	# Abnormal	31	48	25	5	7	–
	Total scored	138	148	110	40	42	–
	% Abnormal	22	25	23	12	17	–
Mitogen	# Abnormal	23	27	17	25	22	–
	Total scored	150	189	100	100	100	–
	% Abnormal	15	14	17	25	22	–
Paclitaxel bolus							
Direct	# Abnormal	15	25	17	14	19	6
	Total scored	50	50	50	45	50	34
	% Abnormal	30	50	34	31	38	18
Mitogen	# Abnormal	31	48	24	39	22	27
	Total scored	92	133	100	100	94	100
	% Abnormal	34	36	24	39	23	27
Paclitaxel infusion							
Direct	# Abnormal	8	5	16	12	11	8
	Total scored	40	36	46	30	35	42
	% Abnormal	20	14	35	40	31	19
Mitogen	# Abnormal	19	21	8	14	16	18
	Total scored	100	100	100	100	100	100
	% Abnormal	19	21	8	14	16	18

the results for each individual animal. For each drug, the panel on the left demonstrates the results obtained when metaphases were scored in samples harvested directly after bone marrow aspiration. The panel on the right summarizes the data examining metaphases harvested from samples cultured in staphylococcal mitogen for 96 h. Fig. 8 depicts the results in the combined direct and the mitogen-stimulated metaphases, and these results are summarized in the Tables 1 and 2. As can be seen in the combined data, with all three drugs the number of abnormal metaphases was statistically higher following bolus administration



	Direct	Mitogen-Stimulated	Combined
Thiotepa	P < 0.0001	P = 0.0007	P < 0.0001
Paclitaxel	P = 0.0933	P < 0.0001	P < 0.0001
Etoposide	P = 0.0035	P = 0.0165	P = 0.0015

p-values were determined using an exact stratified Cochran-Armitage test

Fig. 8. Comparison of abnormal metaphases tabulated both in the direct and the mitogen-stimulated samples. The statistical analysis of the results is summarized in the table at the bottom of the figure. All results were analyzed using an exact stratified Cochran-Armitage test.

Table 2
Example of qualitative and quantitative aberrations seen following etoposide chemotherapy

Sample detail	Total metaphases scored	>1 abnormal	Single abnormality	Chromatid gaps	Chromosome gaps	Uncoiled	Chromatid breaks	Chromosome breaks
Animal 1 etoposide bolus	Direct 78	18	12	13	7	8	19	10
Animal 1 etoposide bolus	Mitogen 100	7	16	5	4	1	10	6
Animal 1 etoposide infusion	Direct 138	14	17	16	5	5	10	2
Animal 1 etoposide infusion	Mitogen 150	8	15	2	5	0	8	6

compared to infusion of the same chemotherapy agent.

4. Discussion

We describe the results of a study designed to quantitate the extent of chromosomal damage in bone marrow cells following chemotherapy. Previous studies have reported schedule dependent modulation of toxicity and efficacy of chemotherapy agents [33–40]. We sought to evaluate the extent of schedule-dependent genotoxicity by administering chemotherapy as either a bolus or a 96 h continuous infusion. Thiotepa, etoposide, and paclitaxel were chosen as the chemotherapy agents due to their distinct mechanisms of action. Within the constraints of a limited sampling strategy we monitored the degree of neutropenia to ensure that comparably cytotoxic amounts of drug had been administered. All three drugs produced more abnormal metaphases following bolus administration compared to a 96 h infusion. We conclude that infusional administration of thiotepa, etoposide and paclitaxel is less genotoxic to normal bone marrow cells than is bolus administration.

A comparative study of the genotoxic effects of these agents on normal primates has not been previously performed. The potential that clastogenic chromosomal damage can be modulated has not been previously addressed in a prospective fashion. There is a paucity of practical data addressing the genotoxic potential of chemotherapeutic drugs, and no recommendations exist on how to reduce this adverse effect. Other studies have examined chromosomal changes in bone marrow and *hprt* mutation frequency in peripheral blood lymphocytes as an index of mutagenic damage caused by exogenous agents such as cigarette smoke and cytotoxic chemotherapy, and whether the ability of individual patients to repair that damage, might predict susceptibility to cancer [41–43]. While these studies have examined more acute effects, we will eventually need to focus on the potential untoward effects of cancer chemotherapy on normal somatic cells leading to secondary cancer and damage to germ cells and fetal tissue; and in turn cancer later in life or even in subsequent generations.

Genotoxic damage is a desirable consequence of cancer chemotherapy if it leads to lethal cytotoxicity in tumors alone. However, increased genetic damage could also have adverse consequences if the affected cell is malignant. Genetic instability characterized by an abnormal number of chromosomes is a common feature of many human cancers and this genetic instability can theoretically be enhanced further by exposure to chemotherapeutic agents [43–47]. Understanding the role of chemotherapeutic agents in inducing genetic damage may help to understand the phenomenon of acquired drug resistance. The Goldie-Coldman hypothesis of drug resistance [48], which evolved from studies in bacteria, proposed that random, spontaneous mutations conferring drug resistance were followed by a positive selection favoring overgrowth of the resistant clone leading to relapse. The alternate possibility that acquired drug resistance could emerge during the course of chemotherapy has been largely ignored. We have shown that drug resistance mediated by P-glycoprotein frequently occurs as a result of a translocation or other chromosomal change which leads to capture of the P-glycoprotein gene by an active gene [17,18]. This phenomenon has been observed in numerous drug resistant cancer cell lines and patient samples [19,20]. Hybrid mRNAs are generated, but because the rearrangement occurs proximal to the start of transcription, the structure of P-glycoprotein remains unaffected. All of these chromosomal aberrations are random and acquired. While the results reported herein involve normal cells, we would surmise that inferences regarding which regimens are less likely to induce drug resistance can be drawn from this study.

Cytogenetically, we concede that the conventional Giemsa staining methods used in the present study are less sensitive than other techniques currently available, including the use of chromosome region specific paints, and especially spectral karyotyping (SKY) [49], since these provide an opportunity to examine sub-lethal and cryptic chromosomal damage in greater detail. While the approach used here provides an accurate estimate of the mutagenic effects of the drugs and regimens used, the majority of gross changes, including breaks, dicentrics, deletions, and ring chromosomes identified by traditional Giemsa staining are likely unstable and lead to cell death. Thus this approach does not provide a suitable means to detect stable heritable changes. The latter include changes such as reciprocal translocations, which are regarded as heritable exchanges. To identify these changes, chromosomal painting and SKY are considered superior. For example, chromosome painting has been used to demonstrate the presence of translocations in the peripheral lymphocytes of Hiroshima atom bomb survivors several decades after exposure [50], and chromosome painting was shown to be a more sensitive method compared with conventional Giemsa-stained metaphase analysis to identify exchange aberrations in human lymphocytes following in vitro exposure to bleomycin and daunomycin [51]. However, the goal of the present study was not to document stable, heritable changes, but rather to compare the relative genotoxicity of different drugs and modes of administration by quantitating the extent of chromosomal aberrations. It was thus felt that using Giemsa staining to score metaphases under a light microscope was suitable for this purpose. The chromosomal aberrations noted in dividing somatic cells can thus be used as a surrogate marker of overall genotoxic potential of a chemotherapy agent.

We hope this study will lead to further investigations to address these issues in a more systematic manner. We believe this is important because as cancer therapy improves and survival is increased, secondary malignancies will become an increasingly important issue. We hope this will also be valuable in addressing

the issue of administering chemotherapy to pregnant women, a group in which the incidence of cancer is increasing dramatically as the age of first and subsequent pregnancies is delayed [52–55]. Finally significant evidence exists that chromosomal alterations are involved in the acquisition of drug resistance, and modulations that impact on this are also likely to yield beneficial treatment outcomes. We believe this approach provides a reproducible model in which drugs, biological agents and eventually regimens can be compared [38–40,56]. The ultimate goal should be the identification of a chemotherapy regimen that is less genotoxic and hence less mutagenic or teratogenic, and hopefully less likely to lead to the development of acquired drug resistance and eventual failure or relapse, while maintaining similar response rates or improving efficacy [58].

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